United States Department of Agriculture
Animal and Plant Health Inspection Service
Center for Veterinary Biologics
P. O. Box 844
Ames, IA 50010

1. Reagent Name: *Clostridium perfringens* type D (epsilon) toxin

2. Strain or Source: Not applicable

3. Lot Number: IRP 450

4. Fill Date: September 13, 1999

5. Expiration Date: No expiration date has been assigned to this product because *C. perfringens* type D (epsilon) toxin has demonstrated over time to be very stable if properly stored. The stability of this reagent will be routinely monitored by the Center for Veterinary Biologics.

Precautions: This reagent does not present a hazard to laboratory personnel who work with the toxin provided sound fundamental laboratory techniques are followed.

6. Intended Use: IRP 450 serves as the standard toxin when conducting *C. perfringens* type D (epsilon) toxin-neutralization tests in mice.

7. Instructions for Use: IRP 450 diluted 1:320 is considered the standard toxin dilution when conducting toxin-neutralization tests in mice as outlined in 9 CFR 113.112 and 9 CFR 113.455. The dilution is prepared by adding 1.0 mL of IRP 450 to 31 mL of peptone diluent (1.0% peptone, 0.25% sodium chloride, pH 7.2). The toxin is further diluted to 1:320 by adding 1.0 mL of the 1:32 dilution to 9.0 mL of diluent. A volume of 0.6 mL of the toxin diluted 1:320 and 0.4 mL of diluent is equivalent to 10 L_o doses. A volume of 0.9 mL of toxin and 0.1 mL of diluent is equivalent to 10 L_+ doses. *C. perfringens* type D (epsilon) toxin IRP 450 diluted 1:32 is stable when stored at -70°± 5°C.

8. Test of Reagent:

Determination of test dose of toxin - The L_o and L_+ doses were established by injecting mice intravenously with 0.2 mL of a mixture of varying amounts of IRP 450 combined with 0.1 unit of standard epsilon antitoxin. The L_o dose for the *C. perfringens* type D (epsilon) toxin neutralization test is the largest amount of toxin which can be mixed with one-tenth unit of standard antitoxin and not cause death in injected mice within 24 hours. The L_+ dose is the smallest amount of toxin which can be mixed with one-tenth unit of standard antitoxin and cause death in at least 80% of injected mice within 24 hours.

Determination of LD_{50} - Harlan Sprague Dowley female mice weighing 16-20 g were injected intravenously with 0.2 mL of toxin diluted in peptone diluent. The toxin was found to contain $10^{5.054}$ mouse lethal dose fifty (LD_{50}) per mL.

Determination of toxin type - The toxin type was confirmed by performing toxin-neutralization tests in mice. The mice were injected intravenously with mixtures of IRP 450 and *C. perfringens* type A, B, C or D antitoxin. All of the mice died within 24 hours except those receiving mixtures containing type B or D antitoxin.

Sterility test - The toxin was tested for sterility and found to be free of viable bacteria and fungi according to procedures outlined in 9 CFR 113.26.

9. Container Size, Type, Weight, or Volume: Three-mL glass vials each containing 1.3 mL of toxin.

10. Storage Conditions: Store at -70°± 5°C.

11. CVB Technical Contact: Bacteriology Section, Center for Veterinary Biologics, (515) 337-6140 or FAX (515) 337-7673.

12. Origin and Passage History: *C. perfringens* type D (epsilon) culture CN3688, used to produce IRP 450, was obtained from Coopers Animal Health, Inc., 1201 Douglas Avenue, Kansas City, KS 66103-1438, on January 5, 1976. The number of passages is unknown.

13. Method of Preparation: Culture CN3688 was grown in a 40-liter New Brunswick fermentor containing media consisting of N-Z case, proteose peptone, and yeast extract. Actively growing culture was aseptically added to the fermentor and incubated at 35°C for approximately 6 hours. The culture was centrifuged and the supernatant passed through a Millipore filtration unit containing a 0.22-µm membrane. The filtrate was further processed using a Millipore pellicon cassette system containing a high volume ultrafilter. The concentrated toxin was adjusted to pH 6.8 and passed through a sterile Millipore filtration unit containing a 0.22-µm membrane. Sterile glycerol was added to the product at a final concentration of 5% v/v.

14. Other:

Reagent orders and feedback should be sent *including phone number* to the following email address: CVB@aphis.usda.gov

Reagent orders forms (APHIS 2018) are available from:
http://www.aphis.usda.gov/animalhealth/cvb_forms

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